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TI Purification of secoisolariciresinol dehydrogenase.

AU Davin, Laurence B.; Xia, Zhi-Qiang; Costa, Michael A.; Fujita, Masayuki; Lewis, Norman G.

CS Inst. Biol. Chem., Washington State Univ., Pullman, WA 99153 USA

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little observed even in the oxidative stress treatment. [1] H. Haraguchi et al. (1995) *Planta Med.* 61: 333-336; [2] H. Haraguchi et al. (1996) *Planta Med.* 62: 217-221; [3] H. Haraguchi et al. (1996) *Phytochemistry* 43: 986-992.

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Session 55, Natural Products, Medicinals, Ethnobotany

Purification of secoisolariciresinol dehydrogenase. Davin, Laurence B. *Institute of Biological Chemistry, Washington State University* Xia, Zhi-Qiang *Institute of Biological Chemistry, Washington State University* Costa, Michael A. *Institute of Biological Chemistry, Washington State University* Fujita, Masayuki *Institute of Biological Chemistry, Washington State University* Lewis, Norman G. *Institute of Biological Chemistry, Washington State University*

The plant lignan, matairesinol, is known to have important roles in the diet in cancer prevention, as well as being a putative intermediate in formation of the plant anti-cancer compound, podophyllotoxin. Matairesinol has also been implicated in plicatic acid formation in western red cedar, suggesting that expression of the pathway might serve as a marker for heartwood formation. The enzyme, secoisolariciresinol dehydrogenase, which catalyses the enantiospecific conversion of (-)-secoisolariciresinol into (+)-matairesinol has been purified from *Forsythia* species. Purification of the enzyme, its catalytic properties and enantiospecificity, and progress towards cloning its gene are described.

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Session 55, Natural Products, Medicinals, Ethnobotany

Ginsenoside synthesis in germinating embryos of American ginseng. Hansen, Brad G. *Department of Biology, University College of the Cariboo, Kamloops B.C., Canada* Smith, Ron G. *Department of Biology, University College of the Cariboo, Kamloops B.C., Canada*

Panax quinquefolius roots have been used for many years in traditional medicine. The ginsenosides, found in ginseng, partly account for ginseng's medicinal effects. The structures of ginsenosides have been elucidated but the pathway leading to their synthesis has not. In this study we investigated the pathway of ginsenoside synthesis by using a pulse-chase experiment. Freshly germinating embryos (~ 0.5 g) were incubated in ¹⁴C radiolabelled acetate (200 kBq/micromol acetate) for 2.5 hr. Following the incubation period some of the embryos were immediately placed in methanol to quench metabolism and extract ginsenosides from the tissue. Three samples were acquired after placing the embryos in unlabelled media for chase periods of 1.5 hr each. Ginsenosides were extracted from the samples at -20°C for 1 wk. The extracts were analyzed for their ginsenoside content by HPLC. Fractions were collected from the HPLC output for liquid scintillation counting and peak identification. The total ginsenoside content (based on seven standard ginsenosides) of the germinated embryos was found to be 0.25 micromol/g tissue. The embryos were found to mostly contain Re, Rd, and two major unidentified ginsenoside peaks (U1, U2); Rg1, Rf, Rb1, Rb2, and Rc were present in minor amounts. By using the data from the initial incubation period we found that the synthetic rate of ginsenosides was 560 nmol ginsenoside/mg fw/hr. About 70% of the label appeared in Re, U1, and U2 after the 2.5 hr incubation. With the chase periods the percent label increased in Re and U2, and disappeared in U1. Minor amounts of label were found in Rg1, Rf, Rb2, and Rd. From these results we conclude that U1 is likely to be the precursor molecule for ginsenoside biosynthesis in germinating embryos.

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Characteristics of hybrids between Jakyungjong(violet-stem) and Hwangsukjong(yellow-berry) in Panax ginseng C.A.Meyer. Choi, Kwang-Tae *Korea Ginseng & Tobacco Research Institute* Kwon, Woo-Saeng *Korea Ginseng & Tobacco Research Institute* Lee, Myong-Gu *Korea Ginseng & Tobacco Research Institute*

Korean ginseng is a perennial medicinal plant that has been used for either geriatrics, tonic, stomachic, or aphrodisiac effects. All of ginsengs which have been cultivating in Korea are Jakyungjong(violet-stem variant with red berry). Jakyungjong is local race with deterior characters. Therefore, a lot of the individual ginseng plants have been selected for developing a new ginseng varieties. Among them, Hwangsukjong was found. Hwangsukjong has yellow berry and green stem. Hwangsukjong was crossed with Jakyungjong to clarify the inheritance of stem color and then the characteristics of F1 and F2 hybrids were investigated. Most of the aerial part characters of F1 and F2 plants were similar to the female plants, while the stem color in F1 generation was violet. In F2 generation, the stem color was segregated in a ratio of 3 violet to 1 green.

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Session 55, Natural Products, Medicinals, Ethnobotany

Isolation of a major soluble protein present in tubers of Oca (Oxalis tuberosa M.J. Flores, Teresita Graduate Program in Plant Physiology, Pennsylvania State University Michaels, J. Paula Dept. Plant Pathology, Pennsylvania State University Hector, H. Flores Dept. Plant Pathology, Biotechnology Institute, Pennsylvania State University

Oxalis tuberosa (Oca) is an Andean tuber-crop cultivated from Venezuela to Argentina between 2000 to 4000 m. In spite of the fact that people of the highlands in South America consume oca on a daily basis, there are no studies on the chemical composition of oca tubers, specifically its nutritional value. We were able to obtain tubers from oca plants grow under hydroponic conditions. After 4 months tubers were obtained, harvested and proteins from these tubers were analyzed. Studies show that there is a major soluble protein present in oca tubers with a molecular weight of 18 kDa. Analysis of the protein solubility indicated that it is an albumin type protein. It was not present in leaves, stems or roots of oca plants suggesting that it is a tuber-specific protein. The protein has a pI of 4.8 and we were able to separate it by an anion exchange, rotophor separation and reverse phase HPLC. Proteins from oca tubers were able to inhibit the in vitro hyphal growth of *Rhizoctonia solani*. The amino acid content of the protein will be analyzed to determine the nutritional value of oca clones. This work was supported by a grant from the McKnight Foundation

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Session 55, Natural Products, Medicinals, Ethnobotany

Characterization of a storage root protein isolated from the Andean crop species *Mirabilis expansa*. Jorge M. Vivanco *The Pennsylvania State University* Brett J. Savary *USDA-ARS* Hector E. Flores *The Pennsylvania State University*

Two highly basic putative storage proteins were found in the root extracts of *Mirabilis expansa*, a tuber crop from the Andean highlands of Peru. Two 29 kDa protein were purified to homogeneity by ammonium sulphate precipitation, cation exchange and C4 reverse-phase column chromatographies. The molecular weight was determined by SDS-PAGE. These two proteins account for 20% of the root soluble proteins and their basic nature was determined through isoelectric focusing. Amino acid sequence and antibody production studies are being carried out to determine its possible relationship with *Mirabilis* antiviral protein (MAP), a ribosome inactivating protein from *Mirabilis jalapa*, which by previous studies was demonstrated to have antiviral and biotechnological potential.

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Session 55, Natural Products, Medicinals, Ethnobotany

Inhibitory effect of the *Mirabilis jalapa* extracts against potato virus infection. Jorge M. Vivanco *The Pennsylvania State University* Maddalena Querci *The International Potato Center* Hector E. Flores *The Pennsylvania State University*

Extracts of *Mirabilis jalapa*, a plant that contains a ribosome inactivating protein called MAP, were tested for their ability to inhibit infection by potato virus X (PVX), potato virus Y (PVY), potato leafroll virus (PLRV) and potato spindle tuber viroid (PSTVd). Root extracts of *M. jalapa* sprayed 24 hours before inoculation with PVX, PVY or PSTVd inhibited infection by almost 100%. The antiviral activity was expressed against mechanically transmitted viruses, but not aphid-transmitted viruses. MAP was purified from root extracts of *M. jalapa* and shown to have a molecular weight of 28 kDa. Purified MAP showed the same antiviral effect as the crude extracts of *M. jalapa*. Western blot analysis using polyclonal antibodies against MAP revealed that the protein occurred at high concentrations in the roots of the white-flowered variety of *M. jalapa*. A protein of similar size to MAP was detected by western blotting in leaf extracts of the Andean edible crop *Mirabilis expansa* and in *Mirabilis multiflora*, a North American food and medicinal plant.

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Session 55, Natural Products, Medicinals, Ethnobotany

Glucosinolate biosynthesis in *Sinapis alba* - *in vitro* production of the intermediate p-hydroxybenzylaldoxime and studies on the distribution of biosynthetic activity in the developing siliques. Du, Liangcheng *Plant Biochemistry Laboratory, Dep. Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark* Hansen, Carsten H. *Plant Biochemistry Laboratory, Dep. Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark* Wittstock, Ute *Plant Biochemistry Laboratory, Dep. Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark* Halkier, Barbara A. *Plant Biochemistry Laboratory, Dep. Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark*